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Award Number: DAMD17-98-1-8617

TITLE: Role of Glutamate Release and Metabotropic Autoreceptors in Seizureogenic Actions of Cholinomimetic Agents

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REPORT DATE: October 2000

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	October 2000	Annual (1 Oct 99 - 30 Sep 00)

4. TITLE AND SUBTITLE
Role of Glutamate Release and Metabotropic Autoreceptors in Seizureogenic Actions of Cholinomimetic Agents

5. FUNDING NUMBERS
DAMD17-98-1-8617

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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; Distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT ***(Maximum 200 Words)***

The purpose of this project is to delineate the potential role(s) of excitatory amino acids (EAA) as mediators of central nervous system (CNS) excitation and seizures produced by centrally-active cholinomimetic agents and to evaluate possible palliative treatments for central cholinomimetic toxicity. The scope of this project entails simultaneous use of neurochemical and electrophysiological approaches that are designed to assess cholinomimetic-induced excitation in the rat CNS. The goals for this report period were: (1) to establish and validate appropriate electroencephalographic (EEG) recording methods for measuring brain activities in anesthetized rats and to utilize these measures as an electrophysiological index of cholinomimetic-induced seizures, (2) to assess the temporal relationship between changes in EEG activity and extracellular levels of hippocampal excitatory amino acids (EAA) in response to systemic treatment or local intracerebral infusion of cholinomimetic agents, and (3) to undertake preliminary studies of the interaction between cholinomimetic agents and metabotropic glutamate autoreceptor ligands on EEG activity and extracellular EAA levels. The first two goals have been achieved insofar as we have demonstrated high-frequency brain wave activity following intravenous injection or intrahippocampal infusion of pilocarpine. Efforts related to the third goal are underway. These studies will continue into year three.

14. SUBJECT TERMS
Glutamate, Brain, Cholinomimetics, Seizures, *In vivo* Microdialysis, Metabotropic Autoreceptors

15. NUMBER OF PAGES
22

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. Z39-18
298-102

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INTRODUCTION

The overall goal of this project is to evaluate the role of excitatory amino acids (EAA) in the brain as possible mediators of central nervous system (CNS) seizures and neurotoxicity that occurs following exposure to centrally-active cholinomimetic agents. While cholinomimetic agents, including the organophosphate (OP) class of acetylcholinesterase inhibitors, cause profound CNS stimulation that is manifest as seizures, convulsions and irreversible neuropathological damage, current treatments to protect against or reverse OP poisoning fail to ameliorate these CNS toxicities. Current evidence implicates glutamate (GLU) and possibly other excitatory amino acids (EAA) in the excitatory and pathophysiological actions of centrally-active cholinomimetics. The overall goals of this project are to critically evaluate the putative role of GLU as a mediator of the central excitatory effects of these agents and to measure directly the ability of metabotropic GLU autoreceptor ligands to protect against and/or reverse the central actions of cholinomimetic agents. The overall approach involves simultaneous measurements of electroencephalographic (EEG) activity and neuronal release of GLU and L-aspartate (ASP) in rats. The latter measure will be carried out with a novel procedure that affords an enhanced capacity to measure extracellular GLU and ASP *in vivo* with a high degree of temporal resolution. By combining this novel technology with simultaneous electroencephalographic (EEG) measures of cerebral neuronal activity, it should be possible to evaluate the possible role of GLU as a mediator of cholinomimetic-induced CNS excitation and to investigate the utility of metabotropic GLU receptor ligands as a means to prevent cholinomimetic-induced CNS stimulation.

BODY

The major tasks accomplished during this report period were: (1) development and validation of an appropriate method to record EEG activity in chloral hydrate-anesthetized rats in a manner that is compatible with simultaneous monitoring of extracellular amino acid transmitters by fast on-line *in vivo* microdialysis; (2) analysis of the temporal relationship between the appearance of high-frequency spiking activity on EEG recordings following pilocarpine treatments (systemic and intracerebral) and changes in the extracellular concentrations of glutamate (GLU) and aspartate (ASP) in the hippocampal formation; and (3) preliminary evaluation of the effects by metabotropic autoreceptor ligands on pilocarpine-induced changes in EEG activity and extracellular levels of excitatory amino acids (EAA) in the hippocampal formation. Detailed summaries of experimental results are provided in the following sections with relevant data depicted in figures and charts located in Appendix I.

Simultaneous Recording of EEG Activity and Measurement of Hippocampal EAA by Fast On-Line Microdialysis in Chloral Hydrate-Anesthetized Rats.

As outlined in our original proposal, a major focus of this study involved the simultaneous measurement of CNS neuronal firing activity and selected neurochemical changes that occur in concert with seizures produced by centrally-active cholinomimetic agents. The ability to measure simultaneously changes in EEG activity and changes in extracellular EAA levels associated with cholinomimetic drug treatments were crucial to test the central hypothesis of our original proposal. Since the microdialysis-based method we developed during the previous year for fast on-line measurements of extracellular EAA within

brain tissues is compatible for use in anesthetized animals exclusively, we were forced to work within the physical and technical constraints imposed by that instrument in order to monitor cortical EEG activity. With the assistance of Dr. Paul Davenport, we have established and validated a method to record brainwave (EEG) activity in conjunction with microdialysis-based monitoring of hippocampal EAA levels. A brief description of this method and representative results are provided below.

All recordings of brain electrical activity were conducted with an encephalograph from Grass Medical Instruments (Model 8-10C). Initial experiments were conducted with surface (scalp) electrodes in order to monitor global EEG activity in untreated anesthetized rats. Subsequent experiments involved electrode placements proximal to the dialysis probe in order to obtain more focal measures of neuronal electrical activity within the vicinity of the dialysis probe. Under deep chloral hydrate anesthesia, monopolar screw electrodes were inserted through parietal bone at three sites (4mm lateral to the longitudinal fissure and 4.5mm (left side), 2.3mm and 4.8mm (right side) posterior to bregma. EEG activity was recorded on a desktop personal computer (Gateway, Inc.) through an AT-MIO-16 data acquisition board at 100-500Hz band frequency. Fourier transforms of electroencephalograms were carried out with software (TS-find) obtained from Dr. R. Mark Wightman at the University of North Carolina (Chapel Hill, NC). Initial recordings of global EEG activity were used to isolate and identify and eliminate artifactual interferences (noise) from other instruments and unrelated sources. Following successful elimination of noise artifacts as well as other minor technical modifications, we were able to monitor spontaneous EEG activity in conjunction with on-line measurements of extracellular EAA levels in the hippocampus. Representative results are shown in Fig. 1 for EEG activity, GLU and ASP levels during a two-min recording period. In addition to these measures of global EEG activity, we measured focal EEG activity via electrodes located more proximal to the dialysis probe. For these studies, the recording electrode consisted of a stainless steel wire (150μm diameter) that was insulated within a fused silica capillary sleeve (250μm, i.d.). Approximately 2mm of wire protruded from the tip of the capillary with insulation removed from the terminal 1mm section. The electrode and microdialysis probe were affixed at a distance of 350μm apart with the electrode tip immediately adjacent to the center of the active zone (dialysis membrane) of the microdialysis probe. The microdialysis probe and affixed recording electrode were implanted stereotaxically into hippocampus as described previously. The EEG was derived from the potential between the recording electrode and an isolated ground and reference electrodes located on the scalp and forelimb, respectively.

In anesthetized rats, EEG recordings exhibited normal properties, including low amplitude spiking with less frequent occurrences of high-frequency spike complexes (Fig. 2A). Recordings appeared to be stable, reproducible across animals and relatively free from interference by electrical noise from the capillary electrophoresis instrument or other equipment. Fourier transform analysis of electroencephalograms from control animals confirmed our preliminary observations insofar as the vast majority of spontaneous spike activity occurred at frequencies below 10Hz (see Fig. 2B). Since the profile for EEG activity in chloral hydrate-anesthetized rats appeared to be consistent with published data from numerous other studies, we felt confident that the technique was working and suitable for use in the next phase of our investigation. Therefore, we initiated our planned studies of the neurochemical and electrophysiological actions of centrally-active cholinomimetic agents.

Temporal Relationship Between the Changes in EEG Activity and Fluctuations in Extracellular EAA Levels Following Systemic or Intracerebral Pilocarpine Administration.

In our original proposal, we had provided a rationale for the use of either direct-acting or indirect-acting cholinomimetic agents as a model for centrally-active organophosphate (OP) agents. The initial focus of these studies was to identify a cholinomimetic agent as well as a route of administration that would produce the most reproducible seizure activity in chloral hydrate-anesthetized rats. Pilot studies were carried out with physostigmine, an indirect-acting cholinomimetic that has been reported to exert strong CNS activity. Physostigmine was selected for these initial trials since its presumed mechanism of cholinomimetic action is more similar to the actions of OP agents than that of the direct-acting cholinomimetics. However, initial trials with physostigmine failed to generate a pattern of consistent and reproducible results. Intramuscular injection of physostigmine (0.5 – 10mg/kg) produced marked salivation, lacrimation, increased bronchial secretions, muscle fasciculations as well as other symptoms that coincide with intense cholinomimetic activity. However, CNS changes following systemic physostigmine treatment exhibited substantial inter-subject variation with rats exhibiting an increase, decrease or no change in extracellular levels of GLU following drug administration (data not shown). Pretreatment with atropine methylbromide (10mg/kg, s.c.) up to 30 min prior to physostigmine treatment caused a marked reduction in symptoms associated with peripheral cholinomimetic activity but did not substantially improve the inter-subject variability with respect to the central effects of this drug treatment. In view of the possibility that inter-subject variability in the extent of physostigmine uptake into brain could account for our results, a series of studies were carried out wherein physostigmine was administered via direct intracerebral infusion. For these studies, physostigmine (10mM) was delivered directly into the hippocampal formation through reverse dialysis via the microdialysis probe. However, as with systemic administration, intracerebral physostigmine infusion failed to elicit reproducible changes in extracellular EAA levels in our model system. While we do not have a clear understanding of the basis for the highly variable actions of physostigmine in our model, it became clear to us that an alternative experimental approach had to be considered.

Systemic Pilocarpine Treatment. Based upon the number of literature reports that have demonstrated the strong seizureogenic actions of pilocarpine, we undertook a series of studies to evaluate the suitability of this direct-acting cholinomimetic as a prototype agent for our studies. Pilocarpine is a naturally-occurring alkaloid that is an efficacious agonist at muscarinic cholinergic receptors and was one of the prototype agents discussed in our original application. However, in view of its distinct mechanism of action, pilocarpine should exert a more limited spectrum of effects owing to the absence of nicotinic receptor stimulation that accompanies the administration of an indirect-acting cholinomimetic agent. Preliminary studies with pilocarpine involved systemic (intravenous) as well as direct intracerebral administration routes. Prior to intravenous pilocarpine administration (50mg/kg), animals were pretreated with the peripheral anti-cholinergic agent atropine methylbromide in order to block autonomic effects at visceral organs. Pilocarpine administration produced a wide spectrum of physiological changes within 1 to 5 min in anesthetized rats, including twitching of whiskers, muscle fasciculations in fore and hindlimbs and excessive salivation. After approximately 20 min, involuntary muscle contractions occurred over the entire body and discharge of bodily fluids was heavy. Despite pretreatment with atropine methylbromide, half of the pilocarpine-treated animals died within one hour of intravenous drug administration.

Survivors exhibited a gradual dissipation of symptoms during the ensuing two hours of recording. EEG activity recorded proximal to the dialysis probe showed a change in brain wave activity consistent with seizures immediately following pilocarpine injection. As shown in Fig. 3, pilocarpine treatment caused the appearance of high-frequency spiking activity coupled with a modest increase in the amplitude of recorded waveforms. The largest activity changes occurred within the first 5 min following pilocarpine administration and gradually returned to near basal activity over the course of the three- to four hour experiments. In view of these changes in EEG activity and the concurrent appearance of physical symptoms described above, we conclude that the cholinomimetic pilocarpine produces seizures in chloral hydrate-anesthetized rats. Despite this strong evidence for a rapid and reproducible seizureogenic action by intravenous pilocarpine, extracellular levels of GLU and ASP failed to exhibit any significant changes. Continuous monitoring of hippocampal EAA levels at 5 s intervals revealed no significant increases nor decreases for a period of 40 min following pilocarpine administration (Fig. 4). Prolonged measurements of EAA were continued up to 2 hr in two animals with no evidence of any change in extracellular levels of GLU or ASP (data not shown). Therefore, despite electrophysiological (EEG) and behavioral (observational) evidence for the occurrence of seizures following intravenous pilocarpine treatment, direct measurements of hippocampal EAA levels provide no evidence for the direct involvement of GLU or ASP. Since EAA levels remain unchanged throughout the entire period during which seizures are evident in our EEG recordings, it appears unlikely that EAA transmitters play a role in the initiation or maintenance of pilocarpine-induced seizures. However, several factors should be considered before this conclusion can be accepted without reservation. First, since these studies have been conducted in anesthetized rats, it remains possible chloral hydrate may suppress the recruitment and activation of excitatory glutamatergic pathways that otherwise would accompany pilocarpine treatment in awake animals. This remains a viable explanation for our results although it is clear that pilocarpine maintains a seizureogenic action under anesthesia. In addition, despite our use of atropine methylbromide to limit peripheral actions by pilocarpine, the drug is still quite toxic in that half of all treated rats died within the first hour. While this high level of mortality was unexpected, it may reflect the interaction among these drug treatments and the anesthetic agent. Nevertheless, in view of this marked toxicity, we were unable to increase the effective dose of pilocarpine beyond 50mg/kg and, therefore, may not have evoked the full complement of CNS effects that normally accompany cholinomimetic intoxication. In view of this, we have also undertaken studies wherein pilocarpine is administered directly into the brain (intracerebral infusion) in order to avoid many of the toxic peripheral actions of this agent (see below). Finally, it is possible that excitatory pathways in other forebrain regions are involved in the generation and /or propagation of pilocarpine-induced seizures whereas hippocampal glutamatergic neurons are spared from this action. This scenario seems unlikely, however, in view of the well-documented and crucial role of hippocampal circuits in a variety of seizure disorders.

Intracerebral Pilocarpine Infusion. In order to avoid the substantial peripheral toxicity associated with systemic drug treatment, a series of studies were carried out based upon direct intracerebral infusion of pilocarpine. In order to evaluate direct local actions by pilocarpine, drug was administered via reverse dialysis through the same microdialysis probe that was used to sample extracellular EAA levels. For all of these studies, pilocarpine was administered at a concentration of 10mM since that level of drug has been used previously and shown to induce seizures and alter extracellular levels of GLU in awake freely-moving

rats (Khan *et al.*, 1999) and to elevate extracellular GLU in anesthetized rats (Millan *et al.*, 1993). There were no obvious physical effects associated with intra-hippocampal pilocarpine infusion although no physiological parameters (heart rate, blood pressure, respiratory rate, body temperature, etc.) were measured. Following pilocarpine infusion, EEG changes were noted although they tended to be reduced relative to changes observed following systemic drug treatments. As shown in Fig. 5, pilocarpine infusion caused a modest increase in spike amplitude (panel B) and an apparent increase in the number of high frequency spike complexes (panel D). In this animal, the shift in EEG activity following pilocarpine infusion was maintained throughout the remainder of the experiment. In general, intra-hippocampal pilocarpine infusion produced a highly reproducible albeit slight increase in the spike frequency of brain waves. However, there were no associated changes in the spike amplitude or appearance of high-voltage spiking in any animals (data not shown). In conjunction with recording of EEG activity, extracellular levels of GLU and ASP were monitored by *in vivo* microdialysis. EAA measurements were carried out continuously for the first 30 min following pilocarpine infusion and at 5-min intervals thereafter. A total of eight successful experiments were carried out using this paradigm and the results are summarized in Figs. 6 and 7. In four of eight subjects, hippocampal levels of both GLU and ASP decreased immediately following pilocarpine infusion (Fig. 6). The nadir occurred within 5 min for both EAA although the absolute decline was smaller in magnitude for GLU (75 ± 4 % of pre-drug level) than ASP (48 ± 5 % of pre-drug level). While a similar reduction in EAA levels was observed in all experimental subjects (Figs. 6 and 7), these animals differed insofar as GLU levels exhibited a spontaneous reversal. As shown in Fig. 6, GLU levels increased following the initial fall and returned to pre-drug levels within 15 min of pilocarpine infusion. Extracellular GLU continued to increase and achieved a stable level of 170 ± 20 % of pre-drug level after 30 min of continuous pilocarpine infusion. By comparison, ASP increased only slightly and returned to only 80 ± 10 % of its original basal level. Monitoring for up to 180 min revealed no additional changes in either GLU or ASP. Despite the close agreement among data from these four animals, different results were obtained in three additional rats that received intra-hippocampal pilocarpine infusions. As shown in Fig. 7, both GLU (55 ± 8 % of basal) and ASP (60 ± 8 % of basal) exhibited an initial and rapid decline following pilocarpine infusion. However, unlike the other rats, these rats exhibited a sustained reduction in GLU levels that persisted for at least 2 hr with no trend toward a spontaneous rebound as described above. In the final experimental subject, GLU and ASP levels declined slightly before returning to pre-drug baseline levels (data not shown). In summary, despite the relatively small sample size of this study, it is clear that intra-hippocampal infusion of pilocarpine elicits a heterogenous response in chloral hydrate-anesthetized rats. At this time, the underlying basis for this heterogeneity is not known. The immediate response to pilocarpine infusion was qualitatively similar (*i.e.*, a moderate decline in extracellular GLU level) in all animals however the latent response exhibits marked inter-subject differences. Once again, it is possible that the unexplained disparity among animals could be related to differences in the depth of anesthesia at the time of pilocarpine infusion. While this possibility cannot be discounted completely, there appears to be no clear relationship between the length of the delay from the last chloral hydrate injection and the effects associated with pilocarpine infusion. In addition to the disparate neurochemical effects of pilocarpine, it is clear from the recorded EEG activity that intra-hippocampal pilocarpine administration does not produce the expected shifts in amplitude and/or frequency that would be consistent with

cholinomimetic-induced seizure activity. Since this administration paradigm has been reported recently to elicit seizures in awake animals (Khan *et al.*, 1999), consideration must be given to possible interference from chloral hydrate anesthesia.

In an effort to enhance the seizureogenic actions produced by intracerebral pilocarpine infusion, rats were pretreated with lithium chloride (125mg/kg LiCl, s.c.) for 4-6 hr prior to pilocarpine treatment. Lithium is known to enhance the susceptibility to and intensity of pilocarpine-induced seizures through a poorly-defined mechanism of action. A total of six animals were tested in the lithium chloride-pretreatment paradigm. Analysis of EEG activity records suggests a modest enhancement of the excitatory effects associated with intracerebral pilocarpine infusion. While four of six animals exhibited a modest shift to higher frequency spiking activity with no amplitude change (similar to pilocarpine infusion alone), two rats exhibited EEG patterns consistent with seizure activity. As shown in Fig. 8, 40 min of continual pilocarpine infusion in lithium-pretreated rats produced high voltage spikes and low voltage frequency shifts in the EEG pattern (Fig. 8C). Individual high frequency spikes appeared followed by trains of spikes lasting 5 -10 s. This pattern became continuous by 1 hr and lasted approximately 40 min. Despite these clear indications of seizure activity, the rats exhibited no outward signs that would be expected to occur in conjunction with these changes in EEG activity in awake animals. Simultaneous measurements of extracellular EAA levels within hippocampus were carried out in these animals and data are shown in Figs. 8 and 9. When compared to levels immediately before pilocarpine infusion, it is clear that GLU and ASP levels were reduced by pilocarpine treatment. Levels of GLU and ASP decreased immediately following infusion of 10 mM pilocarpine, with GLU reaching a minimum of 65 ± 4 % of basal within 5 min and ASP reaching a minimum of 55 ± 10 % within 10 min. After 60 min GLU levels remained depressed at 60 ± 5 % of basal whereas ASP levels rebounded slightly to 73 ± 10 % (Fig. 9). Further monitoring showed no additional change in either transmitter up to 150 min. The decline in EAA levels clearly precedes the altered EEG patterns (Fig. 8B) and appears to have undergone a slight reversal back toward control levels by the time seizure-like patterns have appeared on the EEG (Fig. 8C). Of particular note, there appears to be no significant change in GLU or ASP levels which precedes or coincides with the train of spikes recorded in Fig. 8C. Removal of pilocarpine resulted in a return to basal level for GLU within 5 min and an increase in ASP levels to 150 ± 20 %. It is interesting to note that pilocarpine infusion appears to reduce the variance in baseline GLU levels insofar as the standard deviation of GLU basal levels fell from 8.4 % to 6.7% following pilocarpine administration. While this trend was observed in every rat, at present we have no firm explanation for the underlying basis or possible significance of this change.

KEY RESEARCH ACCOMPLISHMENTS

- ❑ validation of a modified procedure for monitoring EEG activity in anesthetized rats in concert with fast on-line measurement of extracellular EAA levels by in vivo microdialysis.

- confirmation that systemic pilocarpine administration produces changes in EEG patterns and physiological symptoms consistent with seizures in chloral hydrate-anesthetized rats.

- confirmation that systemic pilocarpine treatment does not produce a consistent rise in extracellular levels of GLU or ASP that is temporally correlated with either the generation or propagation of brain seizure activity.

- preliminary confirmation that intracerebral pilocarpine administration can produce EEG changes that are consistent with seizure activity. This action is evident following lithium chloride treatment, although it is not consistent in all subjects.

D. REPORTABLE OUTCOMES

Reportable outcomes for this period include two manuscripts (submitted), one graduate student dissertation and one meeting abstract as listed below.

Bowser, M.T.; and Kennedy, R.T. "High Resolution Monitoring of Amines In Vivo by CE-LIF", submitted to The Analyst.

Witowski, S.R.; Vickroy, T.W.; and Kennedy, R.T. "Regulation of Synaptic Glutamate Overflow in Hippocampus Following Perforant Path Stimulation In Vivo: Evidence for Volume Transmission", submitted to Journal of Neuroscience.

Monitoring Neurotransmitter Amino Acids in Vivo by Microdialysis With On-Line Flow-Gated Capillary Electrophoresis by Steven R. Witowski, 2000, University of Florida.

Monitoring Amino Acid Neurotransmitters In Vivo With High Temporal Resolution Using Microdialysis. R.T. Kennedy, T. Vickroy, S.T. Witwoski, J.E. Thompson, and Brendan Boyd. (Society for Neuroscience, October 1999)

E. CONCLUSIONS

The results of work conducted during the second year are exciting and have produced some unexpected findings. Based upon studies conducted thus far, it is clear that the putative role of glutamate and other excitatory amino acids in cholinomimetic-induced seizures is not as direct as we hypothesized in our original project. Among the key research achievements during the past year (see Section C), several results hold particular significance. The successful implementation and validation of an EEG recording technique that is compatible with our fast on-line in vivo microdialysis instrument was a key and vital accomplishment. With this expanded capability, we are able to measure simultaneously the global electrical activity of the brain and to monitor underlying neurochemical changes. We have now used this dual approach to address the main focus of this project, which involves the possible role of two excitatory amino acid neurotransmitters (glutamate and aspartate) as mediators of seizures caused by cholinomimetic intoxication. For reasons outlined above, we have selected pilocarpine as a prototype cholinomimetic agent for these studies. Our investigations of pilocarpine actions have entailed both systemic (intravenous) administration of this drug as

well as local application (intracerebral infusion) near the presumed sites that give rise to its seizureogenic actions. The results obtained thus far with pilocarpine do not support our original hypothesis insofar as there does not appear to be a direct relationship between enhanced activity of excitatory amino acids and the development of pilocarpine-induced seizures. On the contrary, pilocarpine appears to suppress excitatory transmission as indicated by the reduction in extracellular levels of both glutamate and aspartate. The reduction in excitatory amino acid transmitters has been observed under several pilocarpine treatment paradigms, including those which produce seizure-like activity on EEG recordings. Therefore, while our original hypothesis cannot be discounted with absolute certainty at this time, it is imperative that all plausible alternative explanations (see above) must be addressed.

However, if we assume that subsequent studies lead us to reject our original hypothesis, then it is reasonable to ask what avenues should be pursued. In this regard, we believe that our original plan to evaluate the potential utility of drugs that activate metabotropic glutamate receptors remains a viable and justifiable option. As outlined above, our data implies that pilocarpine attenuates rather than facilitates glutamate-mediated neurotransmission in the hippocampus. Therefore, it may appear to be unnecessary to evaluate the utility of drugs that suppress glutamatergic neurotransmission if, indeed, glutamate plays no role in the genesis of pilocarpine-induced seizures. However, the underlying premise for that line of investigation was based largely upon our studies in the corticostriatal neuronal pathway wherein metabotropic glutamate autoreceptors inhibit glutamatergic synaptic transmission. More recent studies in our laboratory indicate that this autoinhibitory mechanism may not be present or functional in all brain pathways. Indeed, our recent studies in hippocampus have revealed that selected agonists at metabotropic glutamate autoreceptors can increase rather than decrease extracellular glutamate levels. In addition, recent experimental results in our pilocarpine infusion model have indicated that the metabotropic glutamate receptor agonist ACPD can reverse the suppression of glutamate levels associated with pilocarpine infusion. Therefore, while metabotropic autoreceptor ligands may not act in the precise mechanism that we had originally proposed, it is clear that they still hold significant promise as possible treatment strategies for cholinomimetic-induced seizures. In light of this, it is worthwhile to determine whether these agents exhibit any efficacy against the central excitatory effects produced by cholinomimetic agents and, if so, to attempt to delineate the neurochemical basis for this action.

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G. APPENDIX I (Figures)

The figures on the following pages are related to experimental results that are summarized above in the BODY of this report (Section B). The figures are arranged in the same order in which they are discussed in Section B.

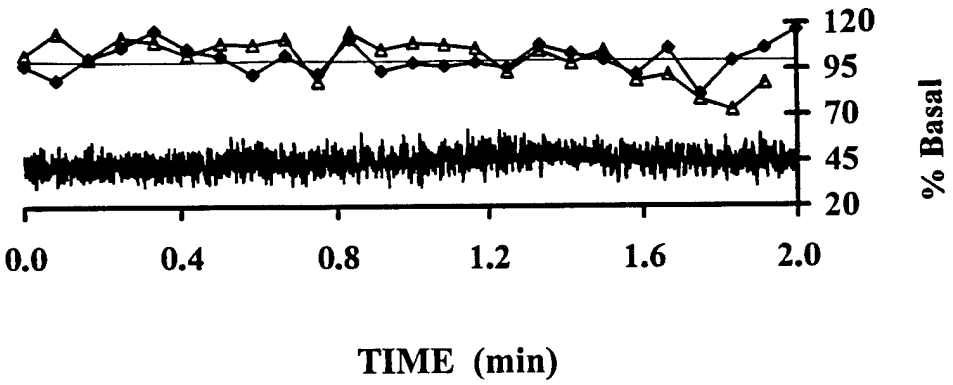

FIG. 1. Representative measurements of GLU and ASP in conjunction with EEG activity in a chloral hydrate-anesthetized rat. Extracellular levels of GLU (diamonds) and ASP (triangles) are plotted as a percent of the basal level. A reference line is shown at 100 percent to demonstrate the temporal fluctuations in these measures. Bottom tracing represent EEG activity measured according to the methods described in the text.

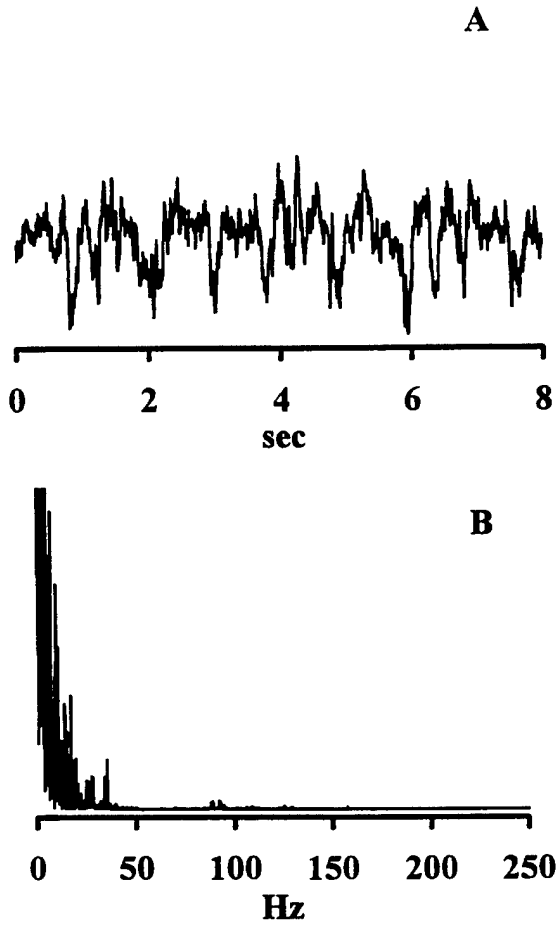

FIG. 2. Representative record of EEG activity in anesthetized rat prior to pilocarpine treatment. Top panel (A) depicts an eight second tracing that contains typical low-amplitude low-frequency spike activity. Bottom panel (B) depicts Fourier transform of data in panel A.

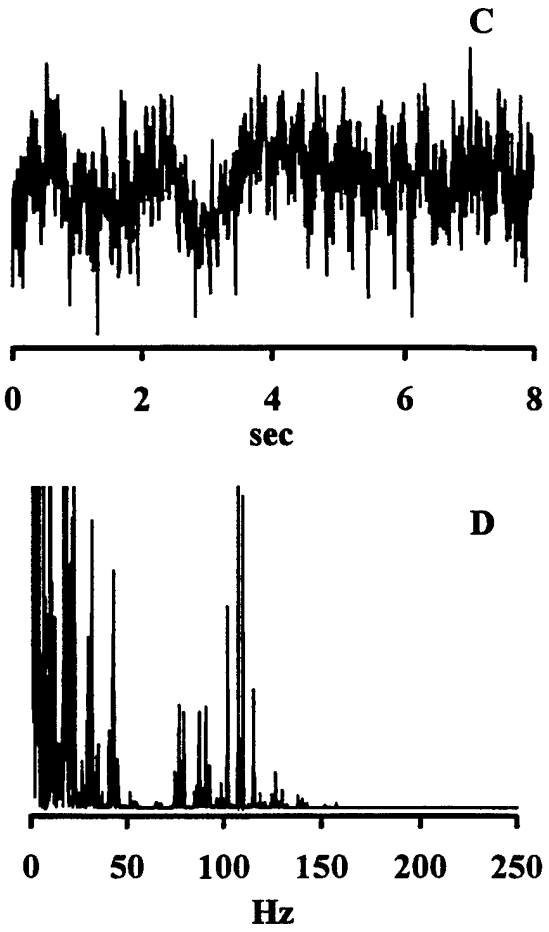

FIG. 3. Effect of systemic pilocarpine treatment on EEG activity. Data are from the same animal as shown in Fig. 2. Activity was recorded 2 min following intravenous injection of pilocarpine. Note the moderate increase in spike amplitude. Fourier transform (bottom panel) reveals marked increase in high-frequency spike complexes compared to control condition.

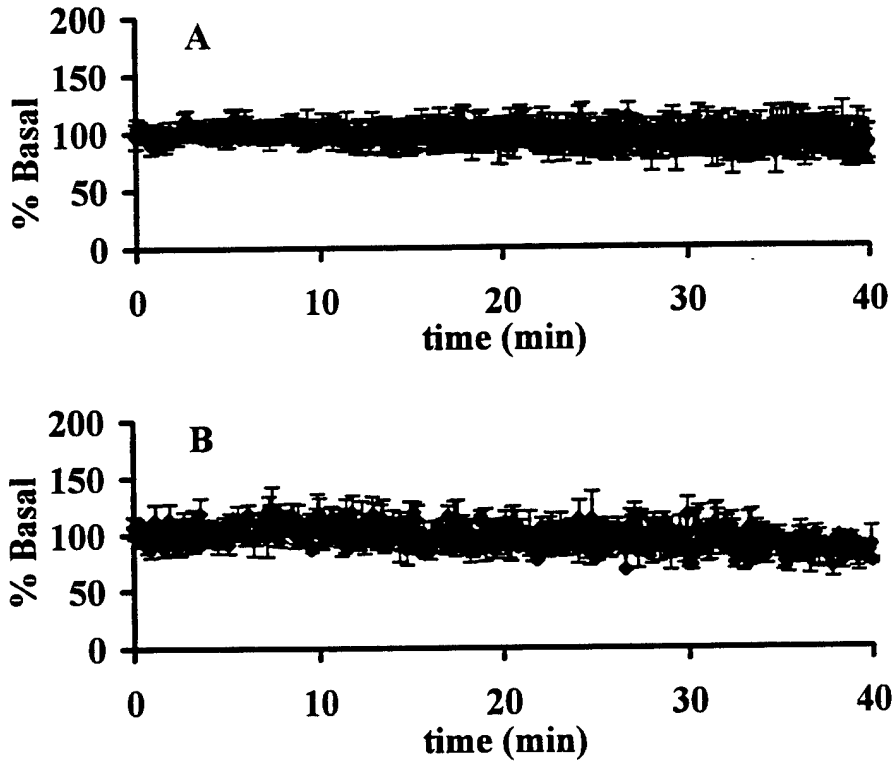

FIG. 4. Effect of systemic pilocarpine injection on extracellular levels of GLU (panel A) and ASP (panel B) in rat hippocampus. Pilocarpine (10 mg/kg) was injected at time zero.

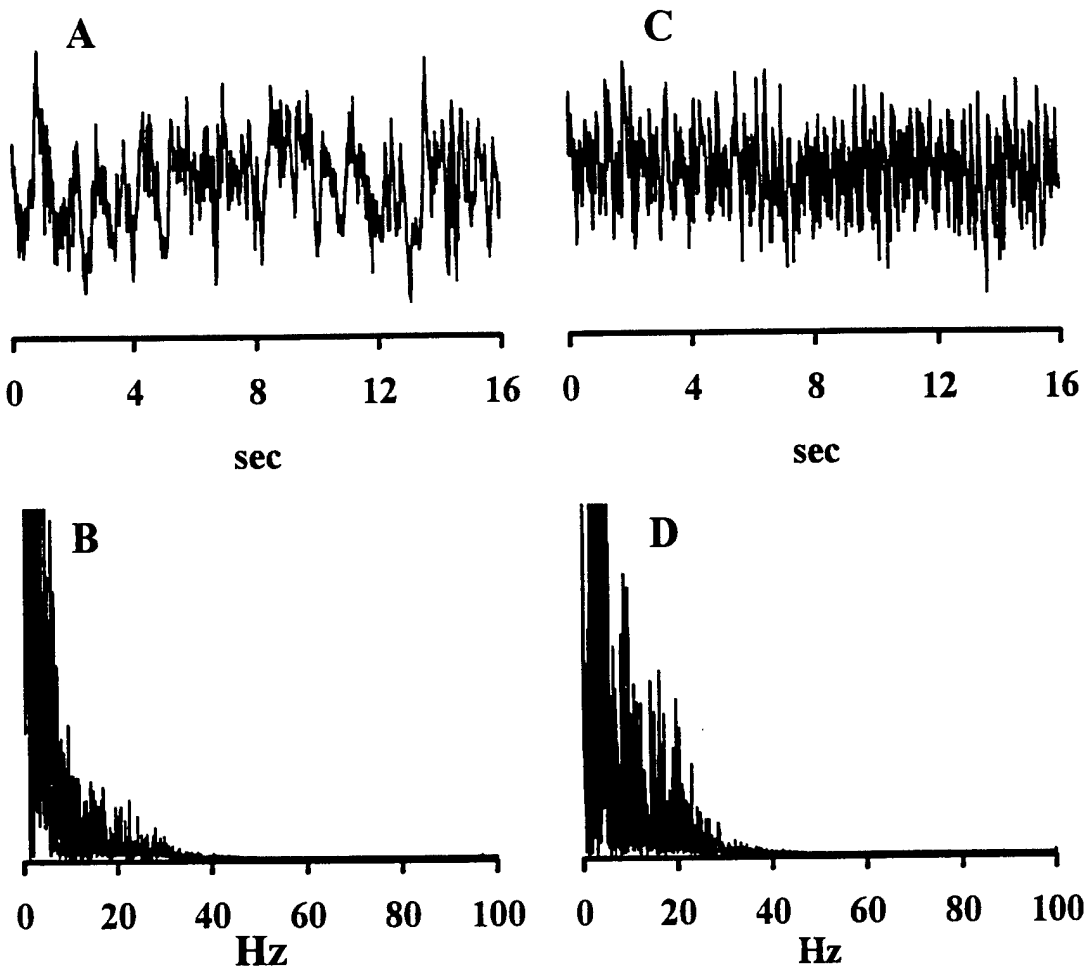

FIG. 5. Effect of intracranial pilocarpine infusion (10mM) on the electroencephalographic activity of chloral hydrate-anesthetized rats. Panels A and B depict EEG activity and a Fourier transform, respectively for the rat immediately before pilocarpine infusion. Panels C and D represent activity immediately following pilocarpine infusion. Data are representative of changes observed in eight rats.

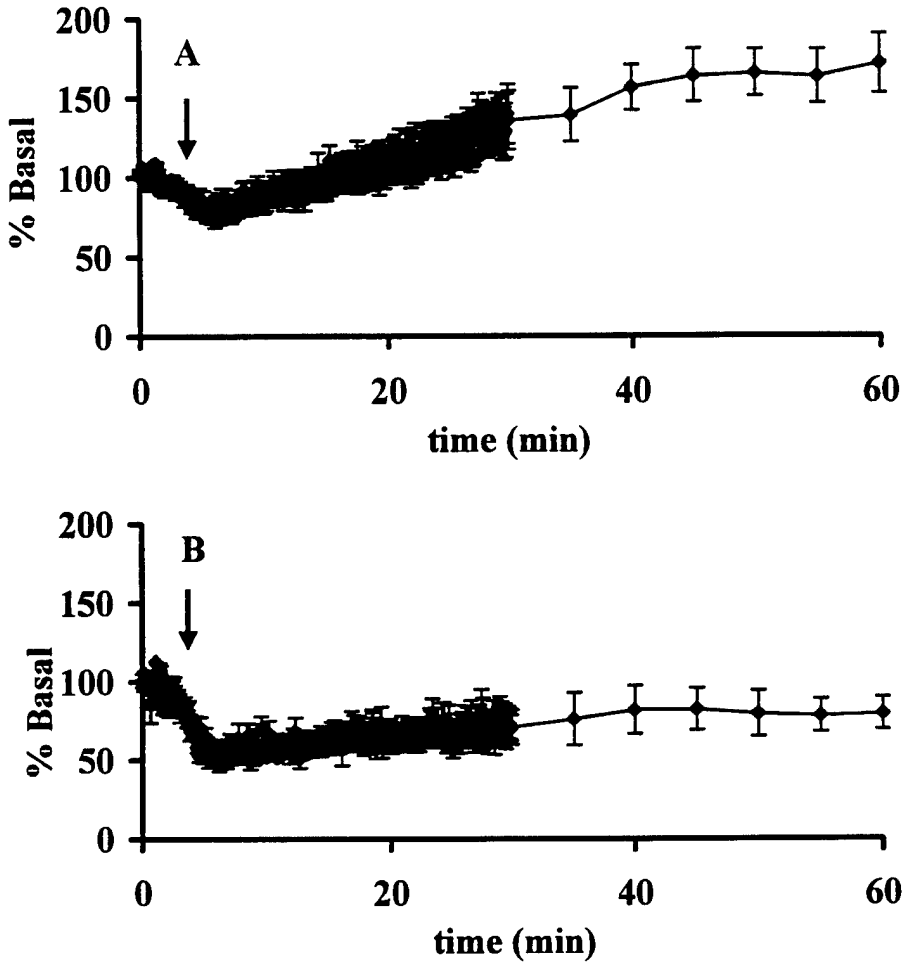

FIG. 6. Effect of intracranial pilocarpine infusion on extracellular levels of GLU (panel A) and ASP (panel B) in rat hippocampus. Arrows indicate the time at which the pilocarpine infusion was begun. Data points represent the means and standard errors for four rats that exhibited a latent rise in GLU concentration.

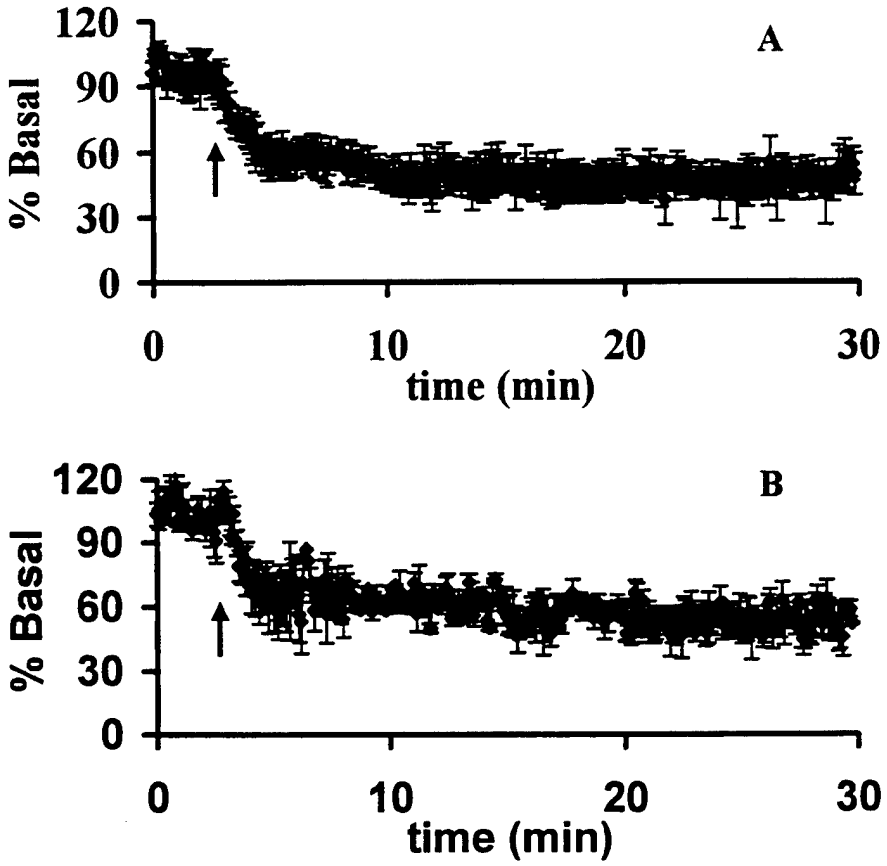

FIG. 7. Same experimental paradigm as described in Fig. 6. Data represent means and standard errors for results from three rats that exhibited a sustained reduction in hippocampal GLU concentration.

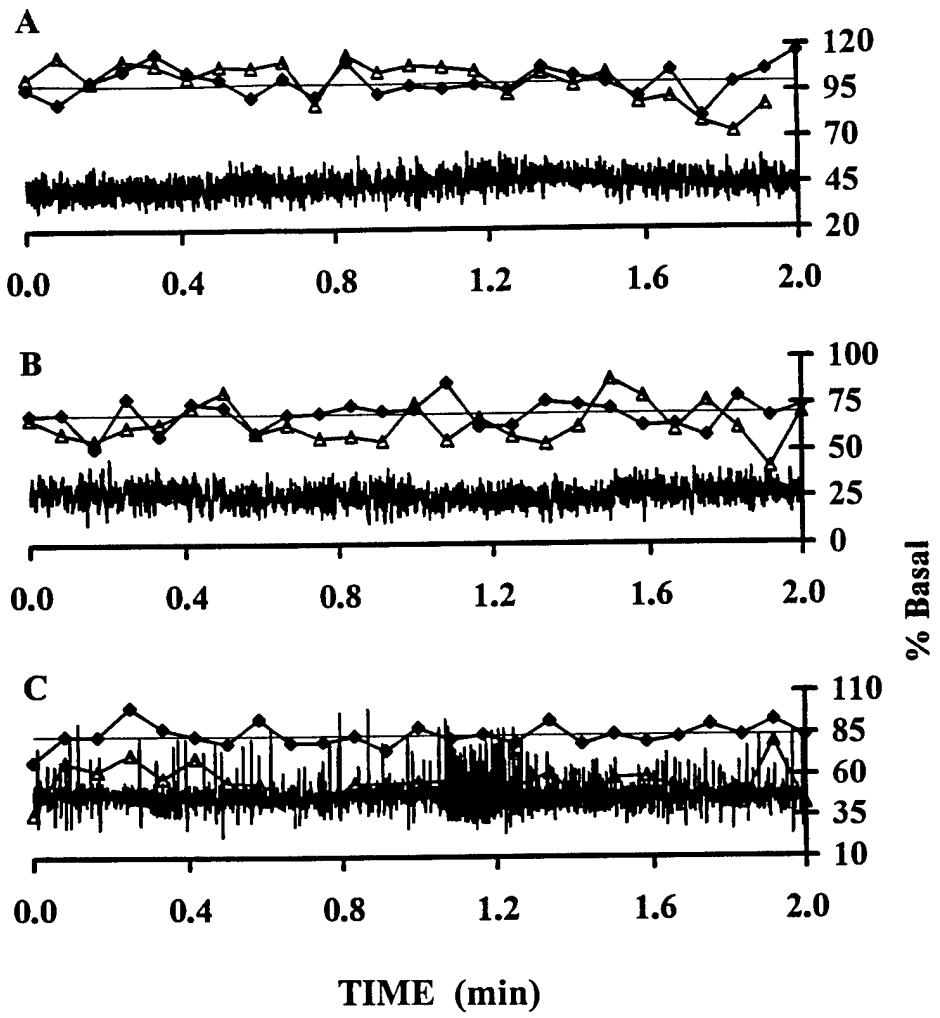

FIG. 8. Simultaneous recording of EEG activity and measurement of hippocampal EAA levels during pilocarpine infusion in lithium chloride-pretreated rats. Extracellular levels of GLU (diamonds) and ASP (triangles) are plotted as a percent of the basal level. A reference line is shown at 100 percent. The continuous tracings at the bottom of each panel represent EEG activity. Each panel represents a two min recording period that was conducted during the pre-drug period (panel A), 20 min following pilocarpine infusion (panel B) or 40 min following pilocarpine infusion (panel C).

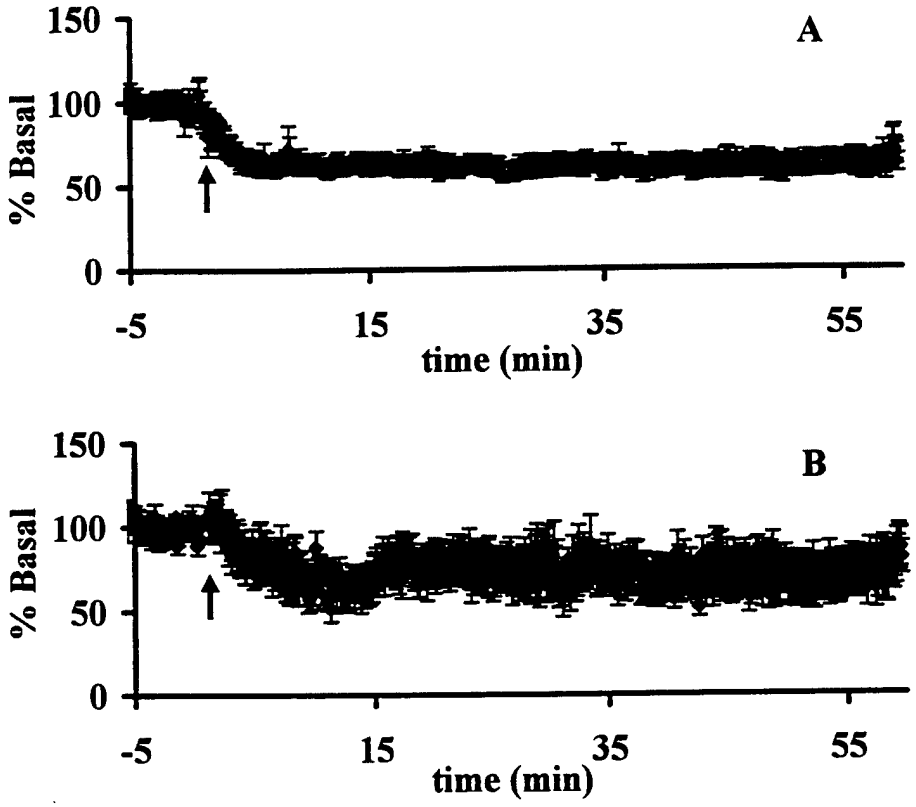

FIG. 9. Prolonged measurement of extracellular GLU and ASP levels following pilocarpine infusion in lithium chloride-pretreated rats. Data for GLU (panel A) and ASP (panel B) represent means and standard errors for all six experimental subjects. Pilocarpine infusion is indicated by arrows.

H. APPENDIX II (Reprints)

The two manuscripts related to this work have been submitted but are not yet in press. Therefore, they have not been attached. If this is in conflict with your guidelines, please contact me and copies will be provided.